Hepatic involvement in congenital cytomegalovirus infection – infrequent yet significant

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SUMMARY. Congenital cytomegalovirus (cCMV) infection can reside in many organ systems; however, the virus has a particular predilection towards inhabiting the reticuloendothelial system, especially the liver. Specific studies focusing only on hepatic involvement in infants with cCMV are lacking. We report our experience with a large cohort of infants treated in our hospital clinic due to cCMV and hepatic involvement. Hepatic involvement was defined either as hepatitis (elevated alanine transaminases (ALT) >80 units/L without cholestatic disease) or cholestatic disease (elevated ALT >80 units/L combined with direct bilirubin >2 mg/dL). During the study period, 198 infants were diagnosed with symptomatic cCMV in our clinic. Hepatic involvement was observed in 13 infants (6.6%); 7 (3.5%) with hepatitis and 6 (3%) with cholestatic disease. Maternal primary infection with cytomegalovirus during pregnancy was diagnosed in 7 (53.8%) of the 13 infants, nonprimary in 3 (23.1%) and unknown in 3 (23.1%). Among these 13 infants, central nervous system (CNS) involvement was observed in 11 (84.6%) and hearing impairment in 7 (53.8%). Treatment with an antiviral agent was initiated in all cases. Gradual improvement of hepatic enzymes and cholestasis was observed over a prolonged period. We found that the incidence of hepatic involvement in infants with cCMV is much less frequent than previously reported. The hepatic involvement in these infants may manifest in two different ways, and thus, a high index of suspicion and a stepwise approach will help in correctly diagnosing these infants. Antiviral treatment due to CNS involvement is warranted and prognosis is excellent.

Keywords: biliary atresia, cholestatic hepatitis, congenital cytomegalovirus, hepatitis, hepatomegaly, idiopathic neonatal hepatitis.

INTRODUCTION

Congenital cytomegalovirus (cCMV) infection is the most common cause of congenital infection and the leading non-genetic cause of sensorineural hearing loss and neurodevelopmental sequelae [1,2]. In infants with symptomatic cCMV, the disease manifestations can range from mild nonspecific findings to multiple organ system involvement, with a particular predilection for inhabiting the reticuloendothelial system [1]. While some studies have reported high rates of hepatobiliary involvement in infants with cCMV manifesting with elevated hepatic transaminases and conjugated hyperbilirubinemia [1–3], specific studies focusing only on hepatic involvement in these infants are lacking. Thus, current data are mostly derived from case reports or studies not involving the hepatobiliary system.

The aim of this study was to report our experience with a large cohort of infants treated in our hospital clinic due to cCMV infection.

PATIENTS AND METHODS

This study was conducted at the Congenital Infection Clinic, Schneider Children’s Medical Center which is the largest paediatric hospital in Israel. The clinical, laboratory and radiological data of all infants with cCMV infection followed in our clinic between January 2005 and December 2013 were retrospectively reviewed.

cCMV infection was diagnosed either by a positive urine culture (shell vial) or polymerase chain reaction (PCR) for cytomegalovirus (CMV) during the first 2 weeks of life; a
positive urine culture or PCR for CMV after birth with a positive PCR for CMV DNA in the infants’ neonatal dried blood spots (DBS); or a positive urine culture or PCR for CMV during the first 8 weeks of life combined with typical findings for cCMV on brain ultrasound (US), funduscropy or hearing assessment.

Additional studies of infants suspected of cCMV included a complete physical examination, including head circumflex; a blood test – complete blood count, liver and kidney function tests; funduscopy performed by a paediatric ophthalmologist; brain US performed by paediatric radiologist; hearing assessment – all infants were examined using brainstem evoked response audiometry (BERA). The BERA thresholds were defined as normal hearing <25 dB, mild hearing loss 25–44 dB, moderate hearing loss 45–69 dB and severe ≥70 dB.

Manifestation of hepatic involvement in infants with cCMV was defined using Boppana et al.’s criteria [3]: (i) hepatitis – elevated ALT >80 units/L without cholestatic disease or (ii) cholestatic disease – elevated ALT >80 units/L combined with direct hyperbilirubinemia defined as direct bilirubin >2 mg/dL. There were no exclusion criteria.

During the study period, all infants with symptomatic cCMV were treated with one of two protocols: (i) intravenous ganciclovir 5 mg/kg/day for 6 weeks followed by oral valganciclovir (Valcyte, Hoffmann-La Roche Ltd, Basel, Switzerland) 17 mg/kg/dose in 2 daily doses for another 6 weeks and then 1 daily dose until 1 year of age, or (ii) oral valganciclovir 17 mg/kg/dose in 2 daily doses for 12 weeks and then 1 daily dose until 1 year of age, as previously described [4].

The study was approved by the Institutional Helsinki Committee.

**Statistical analysis**

Data from each infant were entered into an electronic database and analysed using SPSS 17.0 (Chicago, IL, USA).

**RESULTS**

During the study period, 284 infants were diagnosed with cCMV in our clinic, 198 were considered symptomatic at birth due to central nervous system (CNS) involvement (microcephalus, typical findings in brain US, chorioretinitis or sensorineural hearing loss), and 86 were asymptomatic at birth. Among the 198 symptomatic infants, hepatic involvement was observed in 13 (6.6%), 7 (3.5%) with hepatitis (group 1) and 6 (3%) with cholestatic disease (group 2). Liver function tests and bilirubin levels were within normal range in all 86 infants in the asymptomatic group. Among the 13 infants, only two (one in each study group) were born in our institution; the other 11 were referred to our clinic after birth.

**Prenatal and perinatal diagnosis**

As shown in Table 1, maternal primary infection with CMV during pregnancy was diagnosed in seven (53.8%) of the 13 infants, nonprimary in three (23.1%) and unknown in three (23.1%).

In three neonates from group 1, a prenatal diagnosis of CMV infection was detected intrauterine by positive amniocentesis. In two other cases from group 1, perinatal investigation for various audiological (abnormal newborn hearing screening), laboratory (thrombocytopenia and hepatitis) or radiological (abnormal brain US) reasons led to the investigation and diagnosis of cCMV. A urine culture taken from these five infants tested positive for CMV during the first 2 weeks of life. In the last two cases from group 1, hepatitis detected at 2–8 weeks was the main reason to initiate a comprehensive investigation leading to a diagnosis of cCMV. A urine CMV culture taken from one of these infants tested positive at 4 weeks of age, with an abnormal brain US and hearing impairment. The other infant’s urine culture tested positive at 8 weeks of age with typical findings of cCMV on brain US.

In only one of the six infants in group 2, a prenatal diagnosis of intrauterine infection with CMV was suspected due to maternal seroconversion during pregnancy. In all other cases, an investigation was initiated due to cholestatic jaundice from 2 days old to 8 weeks. Four infants had a positive CMV viral culture during the first 2 weeks of life. One infant had a positive urine culture at the age of 8 weeks with typical findings of cCMV on brain US and positive PCR for CMV DNA in his birth DBS. One infant had a positive urine culture at age 3 weeks with an abnormal brain US and severe hearing impairment.

**Typical cCMV manifestation**

As seen in Table 1, CNS manifestations were observed in all seven infants from group 1, with abnormal findings on brain US in 6 (85.7%) (3 – lenticulostriate vasculopathy, 1 – calcifications, 1 – lenticulostriate vasculopathy and calcifications and 1 – periventricular hyperechosity). These increased rates of CNS involvement in infants from group 1 were also manifested by the fact that 57.1% of these children experienced some degree of hearing impairment. Of the three infants diagnosed with hearing impairment at birth from group 1, one had mild unilateral hearing impairment and two bilateral (1 – severe and mild, 1 – bilateral moderate). One infant with a normal brain US and hearing assessment after birth presented with late onset hearing deterioration and bilateral hearing impairment (moderate and severe) at 4 months old.

Among the six infants in group 2, any CNS manifestations were observed in 4 (66.7%), with abnormal findings on brain US in 2 (33.3%) (lenticulostriate vasculopathy). Hearing impairment was found in three (50%) infants: 1 –

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cases, the scan showed slow excretion of the tracer to the bowel and in another two cases, both dynamic and static images showed tracer retention in the liver and no uptake in the bowel (Fig. 1), compatible with biliary atresia. A liver biopsy was performed on these two children to rule out biliary atresia. In one of these cases, after initiation of antiviral treatment, a haematoxylin–eosin stain on biopsy specimens for CMV identification was negative.

### Treatment and follow-up

Treatment with an antiviral agent was initiated in all 13 cases (groups 1 and 2). Indication for antiviral treatment in the seven infants in group 1 was CNS involvement. Among the five infants from group 1 who were strictly followed-up on their liver function tests after initiation of antiviral treatment, gradual improvement of the hepatic enzymes was observed in all; however, normalization was only noted after 6–19 weeks. Follow-up in our clinic was performed for a period of 19–66 months after birth. Except for one child with mild motor development delay, all are presently healthy and fully developed with normal hearing.

Treatment with an antiviral agent was also initiated in all six cases in group 2. Indication for antiviral treatment in four infants was CNS involvement. However, in two cases, treatment was initiated only due to the severe hepatic involvement. In one of the two infants who showed no excretion into the bowel on the hepatobiliary scintigraphy, a second scan was performed 7 days after initiation of antiviral treatment with valganciclovir, ruling out biliary atresia (Fig. 2). In the four infants from group 1 who were strictly followed-up on their liver function tests after initiation of antiviral treatment, gradual improvement of the hepatic enzymes was observed in all; however, normalization was noted only after weeks and in one case after 1 year. Follow-up in our clinic of infants from group 1 was performed for a period of 12–96 months after birth. The two children with severe hearing impairment at birth had a surgically implanted electronic hearing device inserted (cochlear implant); one had delayed language development. However, all other children are presently healthy and fully developed with normal hearing.

### DISCUSSION

Herein, we report our experience of hepatic involvement in infants with cCMV. While hepatic involvement in cCMV has been described in the past, our report is the first to focus solely on this specific manifestation in a large cohort of infants.

Our main observation was that hepatic involvement was seen in 6.6% of infants with cCMV and might present with two distinct phenomena:

Firstly, hepatitis without cholestasis manifested by elevated hepatocellular enzymes only. In Boppana et al.’s [3] classic study on cCMV, the authors defined the disease

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**Table 1** Clinical data of the 13 infants with cCMV and hepatic involvement

<table>
<thead>
<tr>
<th></th>
<th>Hepatitis</th>
<th>Cholestatic hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Male/female N (%)</td>
<td>4/3 (57.1/42.9)</td>
<td>3/3 (50/50)</td>
</tr>
<tr>
<td>Maternal infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary N (%)</td>
<td>5 (71.4%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>Nonprimary</td>
<td>1 (14.3%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (14.3%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>Time of maternal infection during pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periconceptual or first trimester N (%)</td>
<td>1 (20%)</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>Second trimester N (%)</td>
<td>2 (40%)</td>
<td>–</td>
</tr>
<tr>
<td>Third trimester N (%)</td>
<td>–</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>Unknown N (%)</td>
<td>2 (40%)</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>CNS manifestations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcephalus</td>
<td>1 (14.3%)</td>
<td>–</td>
</tr>
<tr>
<td>Abnormal brain US</td>
<td>6 (85.7%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>Hearing impairment</td>
<td>4 (57.1%)*</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>Chorioretinitis</td>
<td>–</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>Non-CNS manifestations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3 (42.9%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>Purpura</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*One child had late onset hearing deterioration at the age of 4 months.

unilateral mild, 1 – unilateral severe and 1 – bilateral severe.

**Hepatic involvement**

The median and range for liver function tests of the infants in group 1 was 152 (81–484) units/L for ALT and 216 (59–491) units/L for aspartate aminotransferase (AST). Other liver function tests, including cholestatic enzymes and coagulation studies, were normal in all infants in group 1.

Among infants in the second group, median values (and range) for the liver function tests were as follows: ALT 216.5 units/L (90–317), AST 220 units/L (103–403), total bilirubin 10.45 mg/dL (5.3–14.4), direct bilirubin 4.85 mg/dL (4.2–6.3), alkaline phosphatase 734 units/L (345–911) and gamma glutamyl transferase 554 units/L (351–1921). Coagulation studies were normal in all infants. In two cases, the stool was described as acholic. All six infants had a comprehensive investigation including hepatobiliary scintigraphy with Tc-99m DIPA following pretreatment with phenobarbital. The studies consisted of anterior dynamic images during the first 60 min and delayed static images up to 24 h post-tracer injection. The scan was reported as normal in two cases; in two other cases, the scan showed slow excretion of the tracer to the bowel.
course (not only hepatic involvement) in 106 neonates with symptomatic cCMV during 1966–1989 at the University of Alabama, Birmingham, USA. Elevated ALT were the most frequent laboratory abnormality and were observed in 83% of cases [3]. A more recent report from the same centre assessed 178 infants with symptomatic cCMV during 1980–2002 and found hepatitis in 68.3% of 98 screened infants [2]. Another pivotal study was the first to evaluate the effect of ganciclovir therapy in symptomatic cCMV [5]. As 100 infants were enrolled, liver function tests were evaluated only in 82 and ALT ≥100 units/L were found in only 19 (23.2%) infants.

In our report, the incidence of elevated ALT was much lower (6.6%). We believe that in the past few years, the entity of cCMV changed its course mainly due to the awareness of physicians (gynaecologists and paediatricians) as well as pregnant woman. More cases are now suspected or diagnosed during pregnancy and maternal and foetal observation tools are now used, leading to a selected population of infants born with cCMV. For example, among their 106 children described, Boppana et al. [3] reported a very high incidence of severe brain involvement such as microcephaly in 53% and evidence of at least one severe CNS damage in 72%. This very high incidence of a symptomatic infection and these specific signs and symptoms of severe CNS involvement do not usually occur nowadays in developed countries with a high prevalence of maternal US follow-up during pregnancy. The identification of maternal infection during pregnancy and foetal evaluation has led to termination of pregnancy in cases with severe CNS involvement and to the birth of selected less symptomatic infants with cCMV.

The second and more unique manifestation of cCMV is cholestatic liver disease. Two of the above mentioned reports found this manifestation to range between 53.7% and 81% [2,3]. On the contrary, we found that only 3% of our large series cohort exhibited this manifestation. While we used the same definitions as previous studies, we believe that the reason for these differences again reflects the progression which took place in the past few years in identifying and monitoring cCMV, leading to termination of pregnancy in cases of a recognized severely infected foetus.

Fig. 1 Hepatobiliary scintigraphy with Tc-99m DIPAG. The study consisted of anterior dynamic images (a) during the first 60 min and delayed static images (b) up to 24 h post-tracer injection. Both dynamic (during the first 60 min) and static images (up to 24 h post-tracer injection) show tracer retention in the liver and no uptake in the bowel.
We emphasize the fact that when evaluating an infant with cholestatic disease, investigation for cCMV infection should not be overlooked. While some of these infants manifested with cholestatic disease during the first 2 weeks of life, congenital infection can be diagnosed by a simple urine or saliva test. However, at age >2 weeks and due to the fact that a peri- or post-natal infection through genital tract secretions at the time of delivery or through breast milk can result in a positive urine or saliva test, diagnosing congenital infection is more difficult, and consultation with a paediatric infectious diseases specialist is prudent. An antibody test cannot diagnose cCMV and identifying the virus in any body tissue cannot differentiate perinatal from congenital infection of infants aged >2 weeks.

Early identification of cCMV infection in infants with cholestatic disease is very important because as seen in our report, this manifestation may have a lot in common with a main cause of cholestatic disease infants, namely biliary atresia. Biliary atresia is a neonatal disease of the hepatobiliary system, characterized by progressive inflammatory obliteration in the extra- and intrahepatic bile ducts [6]. While it is also the most frequent indication for paediatric liver transplantation, an early, accurate diagnosis is vital because surgery (hepatoportoenterostomy) ensures a higher success rate and better long-term outcome [6,7]. It is important to note that two of our reported children suffered from a clinical and radiological disease strongly suggesting biliary atresia, due to acholic stool, cholestasis and a pathological hepatobiliary scan, which showed tracer retention in the liver with no bowel uptake. Again, a high index of suspicion is needed in these cases to diagnose accurately and treat these infants. Interestingly, previous studies have attempted to connect neonatal cholestasis induced by CMV (perinatal or congenital) to the development of biliary atresia [6,7]. The observation that a progressive fibrosing inflammation of the liver caused obliteration of the bile ducts, led to the theory that biliary atresia may be caused by an immune response to any viral agent, including CMV [8]. Rauschenfels et al. [6] assessed 74 biopsies of infants with biliary atresia for the presence of viral agents and identified CMV only in 8, thus concluding that CMV, as other hepatotropic viruses, does not play
a major role in the aetiology and progression of biliary atresia.

In 2003, Kimberlin et al. [5] used only CNS involvement as an indication for antiviral treatment. In addition, their Phase III randomized and blinded investigation of 6 weeks vs 6 months of oral valganciclovir therapy in babies with cCMV, added hepatitis (elevated transaminases and/or bilirubin) as one of the manifestations for congenital disease and an indication for antiviral treatment [8]. However, one should bear in mind that antiviral treatment will improve hepatic involvement in infants with cCMV. The question whether an infant with cCMV manifested with hepatitis only, without CNS involvement, will be eligible for and will benefit from antiviral treatment is yet to be determined.

In our report, all 13 infants with hepatic involvement were treated and their prognosis was shown to be excellent. However, after initiation of antiviral treatment, only a gradual improvement in the hepatic enzymes was seen and their normalization may occur over a prolonged period of time.

Our study has several limitations. Firstly, this study is retrospective in nature involving a relatively small number of infants with hepatic involvement, making it difficult to establish strong recommendations and conclusions regarding this rare manifestation. Nevertheless, our data do represent the largest cohort of infants reported, because our data have been systematically collected in our hospital clinic during the last decade. It is also important to note that only two infants in our report were born in our centre, reflecting the fact that our centre is a referral centre for cCMV. Dreher et al. [2] proved that referral infants may even overestimate the severity of disease in infants with cCMV because of a selection bias.

Another potential limitation is that our results might reflect our local experience in the field of cCMV. Our data might not fully represent the national or international epidemiology of cCMV due to the high maternal screening for CMV infection during pregnancy in Israel. This may lead to the identification of primary maternal infection and to a compressive workup and close follow-up of every infant with suspected cCMV.

In conclusion, the results of this study indicate that hepatic involvement in infants with cCMV is much less frequent than previously reported and may represent two different manifestations. A high index of suspicion and a stepwise approach will help in properly diagnosing these infants. If antiviral treatment is initiated, a gradual improvement in liver involvement is expected. Larger prospective studies should focus on the hepatobiliary involvement in infants with cCMV to fully understand this unique manifestation.

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STATEMENT OF INTERESTS
Authors’ declaration of personal interests: None.
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